Original Article

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF TINOSPORA CORDIFOLIA IN RODENTS

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ABSTRACT

Context: The currently available drugs for the management of pain, fever and inflammatory conditions presents with many known adverse effects, hence the search for new drugs without adverse effects is required.

Objective: To evaluate the analgesic and anti-inflammatory activities of aqueous extracts of Tinospora cordifolia (AETC) in rodents.

Materials and methods: The leaves of Tinospora cordifolia were identified and authenticated by Botanist. The air dried course powdered leaves were extracted with distilled water and used to evaluate analgesic action by Eddy's hot plate method in mice and anti-inflammatory action by carrageenan induced paw edema in rats. It was tested in five groups of rodents (n=6) for each activity, using 100mg/kg and 200mg/kg of the test drug, 100mg/kg of test drug with 5mg/kg of diclofenac. Diclofenac 5mg/kg and distilled water were used as standard and control, respectively, for both analgesic and anti-inflammatory activities.

Results: Tinospora cordifolia showed significant increase in the reaction time (pain threshold) in doses of 100mg/kg, 200mg/kg, 100mg/kg with 5mg/kg of diclofenac after 30, 60 and 90 minutes of administration. In the same above doses, Tinospora cordifolia showed 32.63%, 36.63% and 40.5% inhibition of paw edema respectively at the end of three hours. With diclofenac the percentage of inhibition was 35.64.

Conclusion: The present study has shown that AETC has significant analgesic and anti-inflammatory activities. The results indicate that identification of active principle from the leaves may add a new, potential analgesic and anti-inflammatory drug to treat acute conditions.

KEY WORDS: Tinospora cordifolia, anti-inflammatory activity, Eddy's hot plate.

INTRODUCTION

Pain and inflammation are common complaints in many patients suffering from acute conditions. They are host defense mechanisms to combat or overcome the invading pathogen or foreign particles. The modern drugs (like opioids, salicylates, corticosteroids) currently used for the management of pain, fever and inflammatory conditions, present with many known adverse effects. Moreover, synthetic drugs are very expensive to develop, on the contrary, many medicinal herbs have been used as therapy for the relief of pain in the past without any adverse effects. [1] It is, therefore, essential that efforts should be made to introduce new drugs which are safer, cheaper and more effective.

There are over 400 different tribal and other ethnic groups in India. Each tribal group has its own tradition, folk language, beliefs and knowledge about use of natural resources as medicines. [2] T.cordifolia finds a special mention for its use in tribal or folk medicine in different parts of the country. Some of the important uses reported in the literature are fever, jaundice, chronic diarrhea, cancer, general debility, cough, pain etc. T.cordifolia is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae. The leaves are membranous and cordate. It is distributed throughout the tropical subcontinent and China, ascending to an altitude of 300 m. Guduchi, the Sanskrit name, means one which protects the entire body. The term Amruthavalli (Kannada), Amtritha (Kashmir) is attributed to its ability to impart youthfulness, vitality and longevity. [3,4,5]

The alcoholic extract of Tinospora cordifolia has been found to exert anti-inflammatory actions in models of acute and subacute inflammation. [6] In another study, aqueous extract of Tinospora cordifolia (AETC) showed a significant anti-inflammatory effect in the cotton pellet granuloma[7] but the acute anti-inflammatory effect studies are sparse. Hence this

study was taken up to evaluate the analgesic and anti-inflammatory activities of AETC in albino mice and albino rats.

MATERIALS AND METHODS

Preparation of extract

The leaves of T. cordifolia were identified and authenticatrd by M. Hanumantha Rao, Botanist, Government College, Manubolu, Nellore. The leaves were thoroughly washed with distilled water, shade dried in dust free environment and made into small pieces, and powdered by grinder. Such 100 gm of powdered leaf material was transferred to a round bottom conical flask, two liters of distilled water was added and soaked for 2 hours, then boiled for 4 hours. The extract was then filtered through filter paper and kept over hot water bath at 50-60°C, until complete evaporation of the solvent and got a yield of 12.8 %(w/w). It was stored at 4°C for future use. [8]

Phytochemical screening

The freshly prepared AETC was subjected to standard phytochemical screening tests for various constituents by standard methods. [9, 10]

Chemicals

Diclofenac (Novartis), Carrageenan (Sigma)

Animals

Swiss albino mice (25-30g) and Wistar rats (150-200g) of either sex were randomly selected from the central animal house at the College and Institutional Animal Ethical Committee clearance was obtained for carrying out the experiment. They were housed in animal room in Air Conditioned atmosphere at an ambient temperature of 25 ± 1° C, with alternative light-dark cycle of 12 h each. They were fed with dry pellets (Sri Venkateshwara Feeds, Bengaluru) and water adlibitum. Animals were deprived of food (but not water), 4 hrs before the experiment. All experiments were conducted between 0900 h and 1700 h.

Analgesic activity

Eddy's hot plate method

Albino mice were divided into five groups consisting of six animals in each.

Group I: 0.5 ml of distilled water, Group II: 5mg/kg of diclofenac,

Group III: 100mg/kg of AETC, Group IV: 200mg/kg of AETC,

Group V: 100mg/kg of AETC + 5mg/kg of diclofenac.

All the preparations were administered orally. Animals were screened by placing them on a hot plate maintained at 55 ± 2° C with the help of thermostat. The reaction time (pain threshold) was noted at 0, 30, 60 and 90 minutes after administration of drugs by oral route.

Anti-inflammatory activity

Carrageenan induced rat paw edema

In this method also rats were divided into five groups of six each. The different groups were pretreated with drugs, similar to Eddy's hot plate method, 60 minutes before injection of 0.1 ml of 1% carrageenan. Carrageenan was injected into the sub plantar region of the right hind paw of rats. The paw volumes were measured using plethysmograph (INCO, Chennai) at 0, 1, 2, 3 and 6 hours after administration of drugs. The average paw swelling in the group of the drug treated rats was compared with that of untreated rats (control group) and the percent of inhibition of the edema was determined using formula

Percent (%) inhibition = 1 – Vt/Vc X 100, where Vt-edema volume in test group, Vc-edema volume in control.

Statistical analysis

Results were expressed as mean ± standard deviation. The statiatical analysis of data was done using one-way analysis of variance (ANOVA) followed by Sceffe's post hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

Preliminary phytochemical screening of AETC leaves revealed the presence of alkaloids, glycosides, diterpenoid lactones and steroids. [11]

Eddy's hot plate method

The results are shown in table 1. Mean reaction time was significantly increased in all the groups in

comparison with control. The test drug groups (III, IV & V) have shown peak effect at the end of 90 minutes. The observations showed that in group V there was maximum increase in reaction time at the end of each 30 minutes in comparison to other groups, which is highly significant ($p < 0.001$).

Carrageenan induced rat paw edema

The AETC and diclofenac, both have significantly inhibited carrageenan induced rat paw edema. Maximum inhibition of paw edema was observed in all the groups at the end of three hours when compared to the control group. Anti-inflammatory activity was expressed as Percent Inhibition (PI). The PI with group II, III, IV and V were 35.64, 32.63, 36.63 and 40.5% respectively (Table 2 and Table 3).

DISCUSSION

The phytochemical screening of AETC has shown the presence of alkaloids, glycosides, diterpenoid lactones sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. [11,12]

The analgesic and anti-inflammatory effects of AETC in various models of pain and inflammation were found to be analogous. The stimulus may be thermal (tail flick, tail immersion and hot plate tests), mechanical (tail or paw pressure tests), electrical (stimulation of paw, tail or dental pulp) or chemical (writhing and formalin tests). [13]

The hot plate method has been found to be suitable for evaluation of centrally acting analgesics. [14]The reaction time in AETC treated groups increased significantly ($p < 0.001$) in comparison to the control group. When AETC in a dose of 100mg/kg with 5mg/kg of diclofenac was given, the mean reaction time was increased more than when these were used separately. All the groups showed the AETC was found to have good analgesic activity in comparison to the control group. Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptor site of pain, while centrally acting analgesics not only raise the threshold of pain, but also alter the physiological response to pain and suppress the patient's anxiety and apprehension. Pain and inflammation are an essential prelude to the repair process. The AETC exhibited potent analgesic effect against thermal noxious stimuli. This is evident as it exhibited good analgesic activity at doses 100mg/kg and 200mg/kg dose as compared to control. This showed that the extract acts as a peripheral analgesic. The analgesic activity is attributed to the reported constituents present in the AETC.

Carrageenan is regarded as an established phlogistic agent/oedemogen, as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility. [15] Edema induced by the sub plantar injection of carrageenan in the rat hind paw is reported to have been inhibited by a number of steroidal and non-steroidal anti-inflammatory drugs. [16]The edema induced in the rat's paw by the injection of 1% carrageenan has a biphasic effect. The first phase is due to release of autacoids, histamine and serotonin (0-2hr), plateau phase is maintained by a kinin like substance which increase the vascular permeability up to two and a half hours. The maximum inflammation is seen approximately three hours post the carrageenan injection (which is attributed to PG release), after which it begins to decline. [17]

In our study the AETC in 100mg/kg, 200mg/kg and 100mg/kg with 5mg/kg diclofenac has shown 32.63, 36.63, 40.5% inhibition of paw edema, respectively, at the end of 3 hrs, while the percentage of inhibition with the standard drug was 35.64. Hence AETC in 200mg/kg showed more anti-inflammatory activity than that of standard drug under the present experimental conditions.

Although elucidating the mechanism of action of T.cordifolia was not the main aim of this study; it is presumed that the analgesic and anti-inflammatory activities are due to the combined effect of various phytoconstituents reported for water extract upon phytochemical investigation. Prostaglandins (PGs) play a significant role in different phases of inflammatory reactions. PG_3 elicits pain by direct stimulation of sensory nerve endings and also sensitizes sensory nerve endings to other pain

provoking stimuli. Since AETC has shown significant analgesic and anti-inflammatory activities, the probable mechanism could be by the inhibition of the PG_3 synthesis.

In conclusion, studies on T.cordifolia are fewer and studies on AETC on analgesic and anti-inflammatory actions are sparse. Hence this study has attempted to fill these lacunae. The standardization of the extracts, identification and isolation of active principles and pharmacological studies of these, needs to be studied further.

A large number of studies on T.cordifolia have showed antiallergic, [18] antidiarrhoeal, antioxidant, [19] antianxiety, antiarthritis, anti-inflammatory, antipyretic, [20] hepato-protective, [21] antimicrobial, antileprotic, antisyphilitic, antigout properties. [3] The present study results have shown the analgesic and anti-inflammatory actions. Further studies on human are needed to prove the safety and efficacy of long term administration of AETC as potential analgesic and anti-inflammatory agent in routine clinical practice.

Groups	Drugs	Dose mg/kg	0 hr	Paw edema in ml			
				1 hr	2 hr	3 hr	6 hr
I	Control	-	0.38 ± 0.06	0.56 ± 0.05	0.69 ± 0.04	1.01 ± 0.05	0.66 ± 0.07
II	Diclofenac	5	0.36 ± 0.03	0.43 ± 0.02***	0.52 ± 0.03 ***	0.65 ± 0.05***	0.75 ± 0.05
III	AETC 100		0.40 ± 0.05	0.45 ± 0.05**	0.54 ± 0.03***	0.68 ± 0.03***	0.80 ± 0.03*
IV	AETC 200		0.37 ± 0.02	0.42 ± 0.02***	0.53 ± 0.03***	0.64 ± 0.02 ***	0.77 ± 0.04*
V	AETC+ 100 + Diclofenac	5	0.37 ± 0.02	0.41 ± 0.02***	0.47 ± 0.02 ***	0.60 ± 0.04***	0.74 ± 0.02

Table 1: Anti-inflammatory activity of the aqueous extract of Tinospora cordifolia by carrageenan induced paw edema in rats

Values are expressed as mean ± SD, n=6 in each group, *p < 0.05, **p < 0.01, ***p < 0.001 as compared to Control group, AETC= aqueous extract of Tinospora cordifolia.

Groups	Drugs	Dose(mg/kg)	1hr	2hr	3hr	6hr
I	Control	-	-	-	-	-
II	Diclofenac	5	21.81	24.63	35.64	15.38
III	AETC	100	18.18	21.73	32.63	23.07
IV	AETC	200	23.63	23.18	36.63	18.46
V	AETC+	100 +	25.45	31.8	40.5	13.84
	Diclofenac	5				

Table 2: Percentage inhibition of paw edema by AETC.

I	Control	-	1.30 ± 0.16	1.28 ± 0.10	1.25 ± 0.15	1.20 ± 0.07
II	Diclofenac	5	2.03 ± 0.34	2.74 ± 0.10***	3.47 ± 0.91***	4.42 ± 1.04***
III	AETC	100	2.21 ± 0.34	2.24 ± 0.56**	2.88 ± 0.26**	3.40 ± 0.49***
IV	AETC	200	2.00 ± 0.46	2.49 ± 0.36**	3.99 ± 0.90***	5.63 ± 0.55***
V	AETC+	100 +	1.94 ± 0.47	2.62 ± 0.57**	4.06 ± 0.70***	5.85 ± 1.20***
	Diclofenac	5				

Table 3: Analgesic activity of aqueous extract of Tinospora cordifolia by Eddy's hot plate method in mice.

Values are expressed as mean ± SD, n=6 in each group, *p < 0.05, **p < 0.01, ***p < 0.001 as compared to control group, AETC= aqueous extract of Tinospora cordifolia.

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